

Application No. 09/626,566  
Filed: July 27, 2000  
Group Art Unit: 1651  
Confirmation No.: 9704

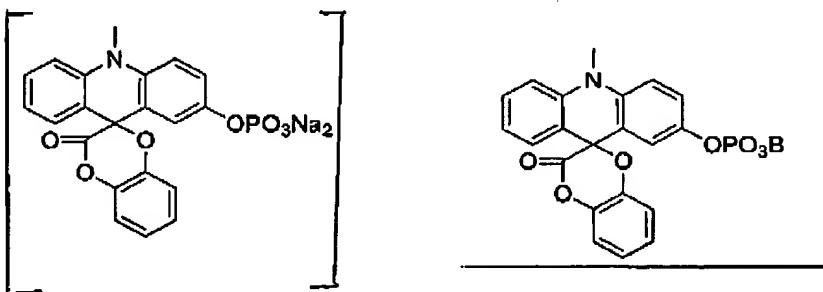
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 43 wherein said counter ions ~~A~~ are ion A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

9-21. (Cancelled)

22. (Currently amended) The chemiluminescent substrate of claim 61 having the following structure:



23-24. (Cancelled)

25. (Cancelled)

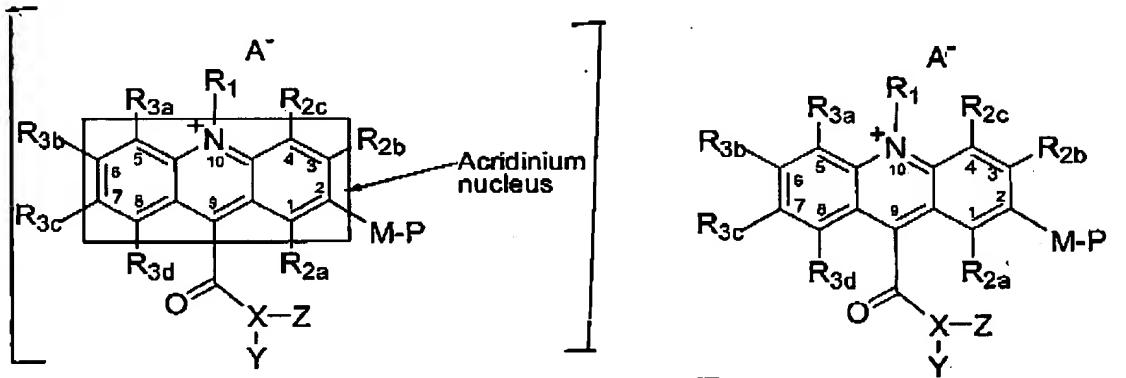
26-28. (Cancelled)

29. (Cancelled)

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30-42. (Cancelled)

43. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is  $\text{PO}_3\text{Na}_2-\text{PO}_3\text{B}$  or a sugar moiety and B is a divalent cation  
or two monovalent cations selected from the group consisting of  
 $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{Mg}$ ;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl,  
 sulfopropyl and sulfobutyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub> are hydrogen;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group

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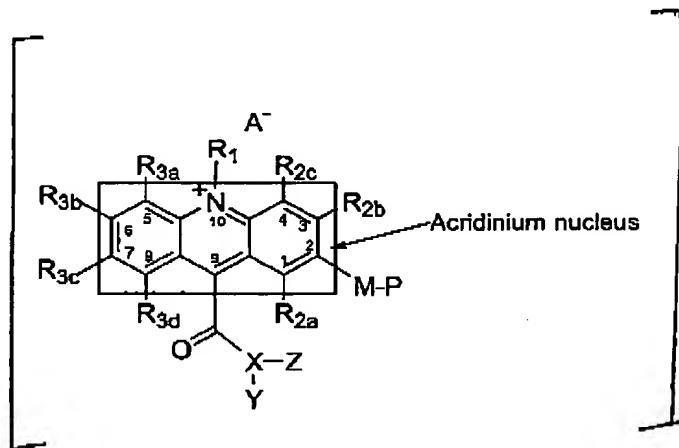
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

44. (Currently amended) A-The chemiluminescent substrate of claim 43, wherein a hydrolytic enzyme, said substrate having the structure,



wherein

P is PO<sub>3</sub>B PO<sub>3</sub>Na<sub>2</sub> or a sugar moiety;

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M is oxygen;

R<sub>4</sub> is selected from the group consisting of methyl,  
sulforpropyl and sulfobutyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub>, are hydrogen;

A<sup>-</sup> is a counter ion for the electroneutrality of the  
quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being  
present if said R<sub>4</sub> substituent contains a strongly ionizable group  
that can form an anion and pair with the quaternary ammonium  
cationic moiety; and

X is O; Y is selected from the group consisting of phenyl,  
(2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-  
4'-carboxyl)phenyl; and Z is omitted.

45. (Currently amended) The chemiluminescent substrate of claim  
43, wherein

P is PO<sub>3</sub>Na<sub>2</sub>PO<sub>3</sub>B;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

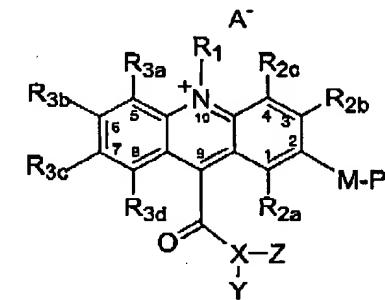
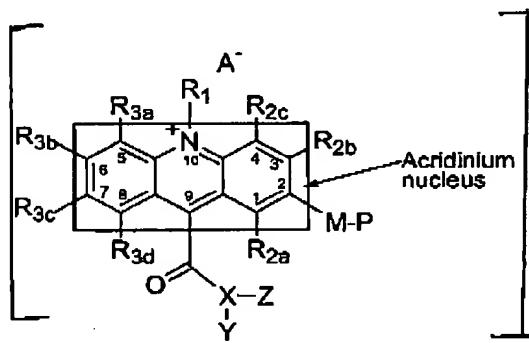
46. (Currently amended) The chemiluminescent substrate of claim  
43, wherein

P is PO<sub>3</sub>Na<sub>2</sub> PO<sub>3</sub>B;

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X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is  $\text{PO}_3\text{Na}_2$ — $\text{PO}_3\text{B}$  or a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of  $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{Mg}$ ;

M is oxygen;

$R_1$  is selected from the group consisting of methyl, sulfoalkyl and carboxymethyl;

$R_{2a}$ ,  $R_{2b}$ ,  $R_{2c}$ ,  $R_{3a}$ ,  $R_{3b}$ ,  $R_{3c}$  and  $R_{3d}$  can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano ( $-\text{CN}$ );

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A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N ~~or~~ and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

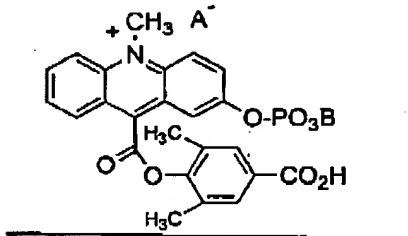
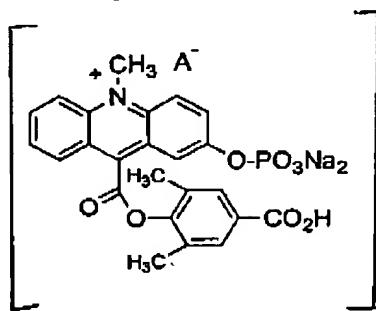
48. (Currently amended) The chemiluminescent substrate of claim 47 wherein said counter ~~ions~~ ion A<sup>-</sup> ~~are~~ is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

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49. (Cancelled)

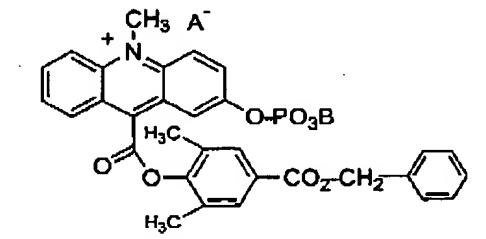
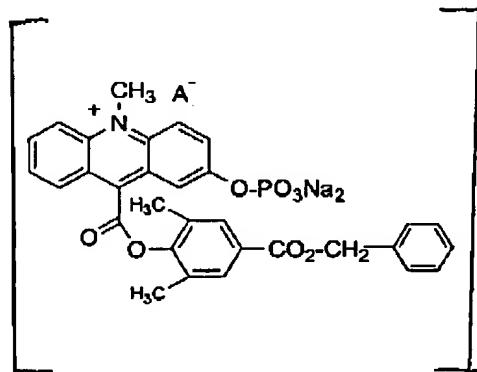
50. (Cancelled)

51. (Currently amended) The chemiluminescent substrate of Claim  
claim 43 -having the structure,



wherein  $A^-$  is selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$  and  $NO_3^-$ .

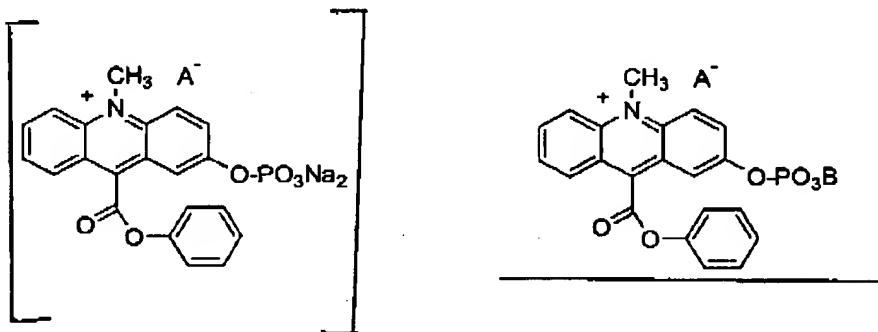
52. (Currently amended) The chemiluminescent substrate of Claim  
claim 43 having the structure,



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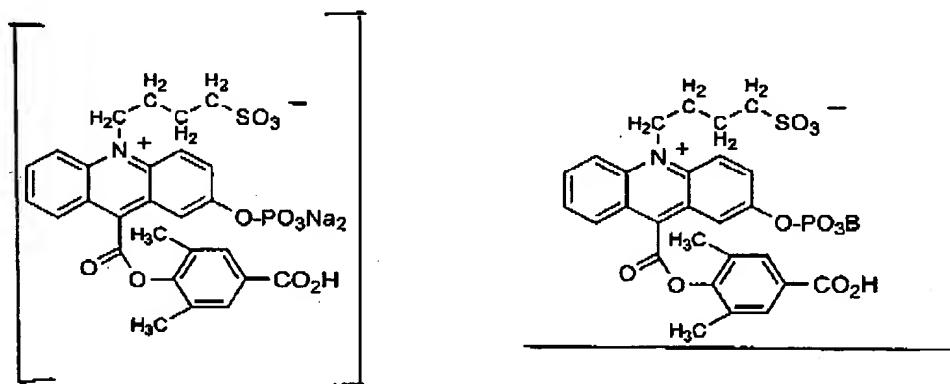
wherein A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

53. (Currently amended) The chemiluminescent substrate of Claim claim 43 having the structure,-



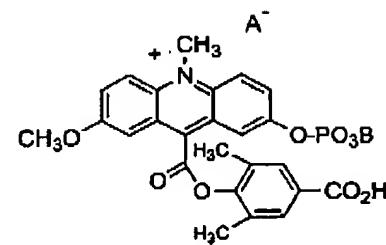
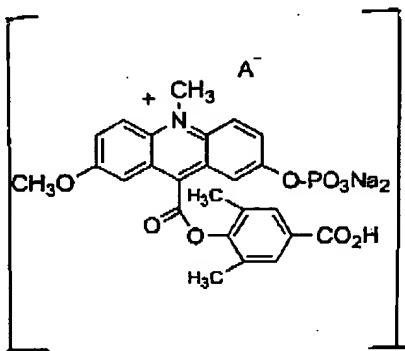
wherein A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup> and NO<sub>3</sub><sup>-</sup>.

54. (Currently amended) The chemiluminescent substrate of Claim claim 43 having the structure



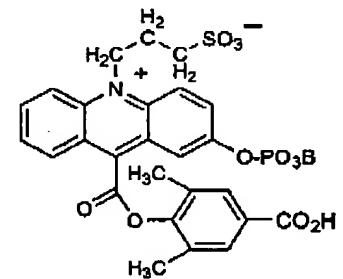
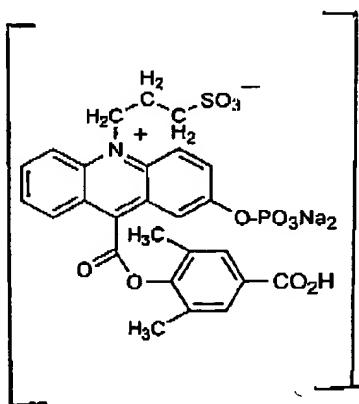
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55. (Currently amended) The chemiluminescent substrate of Claim  
claim 47 having the structure,



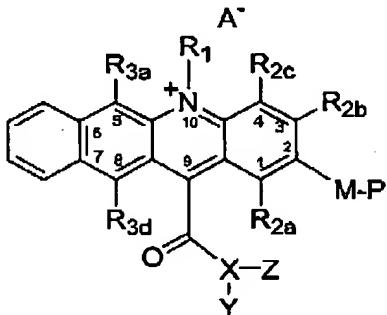
wherein  $A^-$  is selected from the group consisting of  $CH_3SO_4^-$ ,  $FSO_3^-$ ,  $CF_3SO_3^-$ ,  $C_4F_9SO_3^-$ ,  $CH_3C_6H_4SO_3^-$ , halide,  $CF_3COO^-$ ,  $CH_3COO^-$  and  $NO_3^-$ .

56. (Currently amended) The chemiluminescent substrate of Claim  
claim 43 having the structure



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57. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is  $\text{PO}_3\text{B}$  or selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of  $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{MgC}(-\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfebutyl, sulfoalkyl, and carboxymethyl; R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, and R<sub>3d</sub> can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

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A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

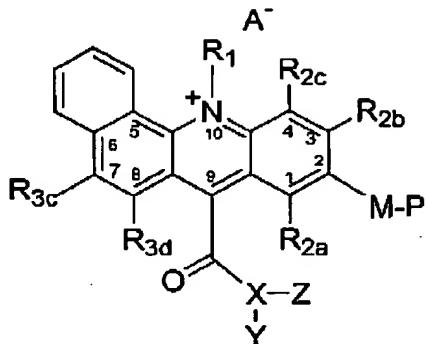
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

58. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

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wherein

P is  $\text{PO}_3\text{B}$  or selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  
 $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and B is a  
divalent cation or two monovalent cations selected from the group  
consisting of  $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{MgC}(-\text{O})\text{R}$  group wherein R is an  
alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl,  
sulforpropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$  can be the same or different, and  
are selected from a—the group consisting of hydrogen, methyl,  
methoxy, halides, and cyano ( $-\text{CN}$ )—;

$\text{A}^-$  is a counter ion for the electroneutrality of the  
 quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being  
 present if said  $\text{R}_1$  substituent contains a strongly ionizable group

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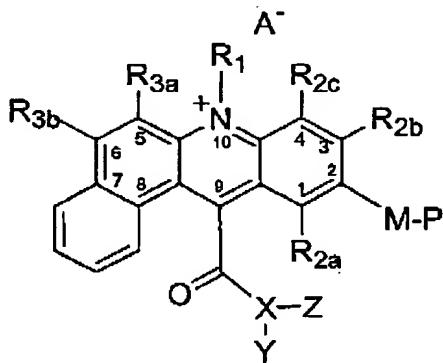
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

59. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



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wherein

P is PO<sub>3</sub>B or selected from the group consisting of PO<sub>3</sub>H<sub>2</sub>, PO<sub>3</sub>K<sub>2</sub>, PO<sub>3</sub>(NH<sub>4</sub>)<sub>2</sub>, PO<sub>3</sub>Ca, PO<sub>3</sub>Mg, PO<sub>3</sub>Na<sub>2</sub>, a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na<sub>2</sub>, H<sub>2</sub>, K<sub>2</sub>, Ca and Mg(-O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub> and R<sub>3b</sub> can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, -halides, and cyano (-CN);

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

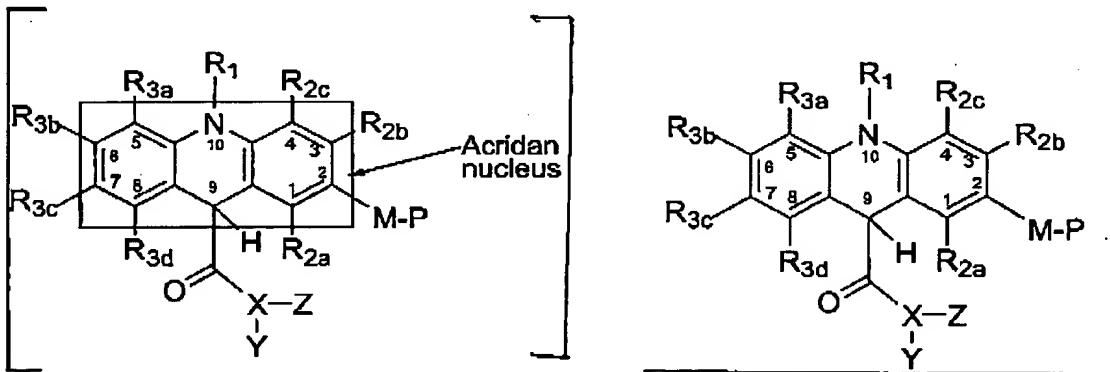
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-

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dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

60. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is  $\text{PO}_3\text{B}$  or selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of  $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{MgG}(-\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

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M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl,  
sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub> can be the same or  
 different, and are selected from a the group consisting of  
 hydrogen, methyl, -methoxy, halides, and cyano (-CN);

A<sup>-</sup> is a counter ion for the electroneutrality of the  
 quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being  
 present if said R<sub>1</sub> substituent contains a strongly ionizable group  
 that can form an anion and pair with the quaternary ammonium  
 cationic moiety; and

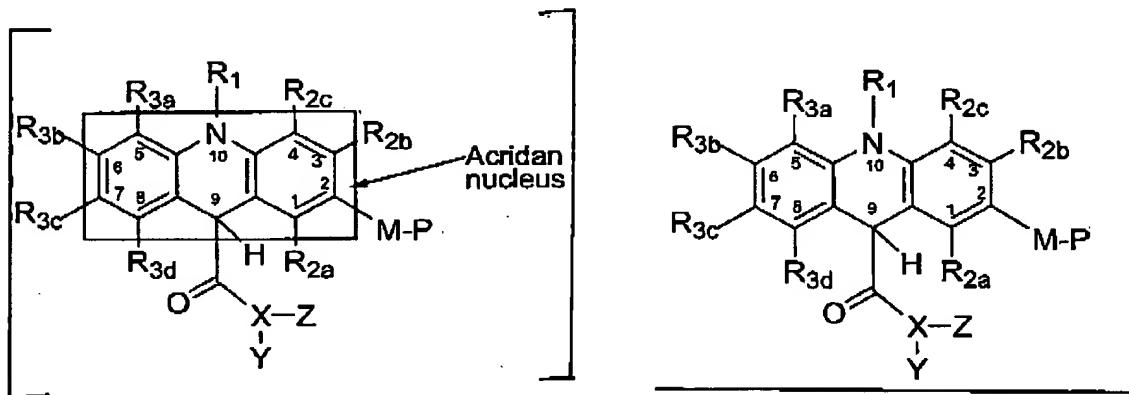
X is selected from the group consisting of O, N or and S,  
 such that,

when X is O or S, Y is selected from the group consisting of  
 phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-  
 dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-  
 benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-  
 benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-  
 benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,  
 (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-  
 carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

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61. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is  $\text{PO}_3\text{B}$  or selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  
 $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of  $\text{Na}_2$ ,  $\text{H}_2$ ,  $\text{K}_2$ ,  $\text{Ca}$  and  $\text{MgC}(-\text{O})\text{R}$  group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfebutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub> can be the same or different, and are selected from a the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

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R<sub>11</sub> is selected from the group consisting of hydrogen, -R,  
substituted or unsubstituted aryl, halides, nitro, sulfonate,  
sulfate, phosphonate, -CO<sub>2</sub>H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR,  
-SSR, -C(O)R, -C(O)NHR, ethylene glycol and polyethylene glycol,  
where R is an alkyl group having 1 to 6 carbon atoms;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X<sub>1</sub> —and X<sub>2</sub> —are the same or different and are selected from the group consisting of O, N or S, such that,

when at least one of X<sub>1</sub> —and X<sub>2</sub> are is O or S, R<sub>11</sub> is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, -CO<sub>2</sub>H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol, or polyethylene glycol, where R is as defined above, and the corresponding Z<sub>1</sub> —and or Z<sub>2</sub> are is omitted; and

when at least one of X<sub>1</sub> —and X<sub>2</sub> is N, the corresponding Z<sub>1</sub> and or Z<sub>2</sub> are is hydrogen, alkyl, aryl or toluenesulfonyl, and R<sub>11</sub> is carboxypropyl.